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SHORT COMMUNICATIONS

Glutathione S-transferase activity towards benz[*a*]anthracene 5,6-oxide in the liver, kidney and lung of cynomolgus monkeys

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Glutathione S-transferases (EC 2.5.1.18) are mainly cytosolic enzymes that catalyse the conjugation of the biological nucleophile glutathione (GSH) with many electrophilic agents (for recent reviews, see refs. 1–3). Several studies, usually in rodents, have shown that GSH S-transferase activities towards a range of electrophiles are generally greater in hepatic than in extrahepatic tissues [3–6]. An exception to this statement is the report by Grover [7] that GSH S-transferase activity towards the K-region epoxide benz[*a*]anthracene 5,6-oxide was more than 2-fold greater in rat lung than in rat liver preparations, but this was not observed by others [8]. It was, therefore, appropriate to determine whether the former finding [7] could be confirmed in another species, such as the monkey. The results obtained are reported in this paper.

Glutathione (reduced) was obtained from Schwarz-Mann, Orangeburg, U.S.A. and ^{3}H–benz[*a*]anthracene 5,6–oxide (sp. act. 7.7 mCi/mmole) was synthesized [9].

Adult male cynomolgus monkeys of body wt. range 5–7 kg were obtained from their natural environment and maintained on a complete dry diet, supplemented daily with fresh fruit. Drinking water was available *ad lib.* to which blackcurrant juice and vitamin B_{12} were added once a week. The animals, which were control animals being used for other studies, were killed by intravenous administration of sodium pentobarbitone, and samples of liver, kidneys and lungs were removed and a post - 105,000 *g* soluble fraction prepared by differential centrifugation [10]. The soluble fraction was dialysed overnight against 200 vol. distilled water, pH 7.0, at 4°. The dialysed tissue supernatants were stored at −20° until required for measurement of GSH S-transferase activity by the method of Grover [7], using 8 mM GSH, 0.08 mM ^{3}H–benz[*a*]anthracene 5,6-oxide (in 0.1 ml of ethanol) and the dialysed tissue supernatant (1 ml) in 0.1 M orthophosphate buffer, pH 7.4 (total volume, 6.1 ml). After incubation at 37° for 1 hr, followed by precipitation of protein by addition of acetone (5 ml), portions (20 μl) of the aqueous supernatant were chromatographed (t.l.c. on silica gel F_{254} plates) using a solvent system of butanol–glacial acetic acid–water (2 : 1 : 1, v/v). Authentic S-(5,6-dihydro-6-hydroxybenz[*a*]anthracen-5-yl) glutathione, prepared by the method of Boyland and Sims [11], was co-chromatographed as a reference compound with the incubation supernatants. The reference compound was located under u.v. light and the corresponding areas of silica gel removed into scintillation vials. The radioactivity adsorbed onto the silica gel was eluted with aqueous ethanol (1 : 1, v/v, 1.0 ml) and measured in a toluene:Triton X-100-based scintillator gel [12].

Protein concentrations of the dialysed tissue supernatants were estimated by the method of Lowry *et al.* [13], using bovine serum albumin as a standard.

Table 1. Glutathione S-transferase activity towards ^{3}H-benz[*a*]anthracene 5,6-oxide in the dialysed tissue supernatants of cynomolgus monkeys

Tissue*	Activity†
Liver	11.0 ± 1.5
Lung	19.7 ± 0.6‡
Kidney	12.3 ± 0.5

* Hepatic aryl hydrocarbon hydroxylase activity in these animals measured by standard procedures (e.g. ref. 22) was 0.38 ± 0.02 nmole equivalents of 3-hydroxybenzo[*a*]pyrene produced/mg protein/min ± S.E.M.: that in the corresponding lung or kidney was generally near to detection limits, about 0.0001 (units as above).

† Activity expressed as nmoles S-(5,6-dihydro-6-hydroxybenz[*a*]anthracen-5-yl) glutathione formed/hr/mg protein ± S.E.M. (N = 10).

‡ Activity significantly greater (analysis of variance $P < 0.001$) than that in liver or kidney.

The results obtained show that GSH S-transferase activity towards the K-region epoxide, benz[*a*]anthracene 5,6-oxide, in the cynomolgus monkey, as in the rat [7], is greater in lung preparations than in those of the liver or kidney, in which the activities of the two tissues were similar. The greater GSH S-transferase activity in the lung compared to that in the liver has not been observed for other polycyclic aromatic hydrocarbons studied (mainly in rodents), such as naphthalene 1,2-oxide [14] or the K-region epoxides benzo[*a*]pyrene 4,5-oxide [8, 15], benzo[*a*]pyrene 11,12-oxide [8], dibenz[*a*,*h*]anthracene 5,6-oxide [8] and 3-methylcholanthrene 11,12-oxide [16, 17], towards which activity in the liver exceeded that in the lung and usually in the kidney, sometimes by several-fold. Similarly, hepatic GSH S-transferase activities towards other electrophilic epoxides such as styrene oxide [18, 19], and non-epoxides, such as 1,2-dichloro-4-nitrobenzene [19], exceeded those in extrahepatic tissues. A review of earlier work [3] provides the same conclusion.

Recent studies [e.g. 20] indicate that K-region epoxides are probably not the ultimate carcinogenic forms of polycyclic aromatic hydrocarbons. One possible contributory reason for this could be the ability of the GSH S-transferase system to metabolize K-region epoxides more readily than non-K-region epoxides [e.g. 8, 21] to relatively less toxic products.

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Factors affecting haem degradation in rat brain

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A functional deficiency of brain haemoproteins has been postulated as a cause of the neural manifestations of acute porphyria [1]. This deficiency could result either from impaired haem synthesis or, alternatively, from increased haem degradation in neural tissue. Factors that are known to precipitate acute attacks might conceivably enhance haemoprotein turnover in brain and thereby deplete the neural haem pool. The object of the present study was to investigate the rate of haem metabolism in normal mammalian brain and in the brains of animals subjected to treatments known to influence this process in liver tissue.

Female Wistar rats (150–230 g) were injected with [4-^{14}C]ALA* (5 μCi/30 μl) intraventricularly [2] and [G-^{3}H]ALA (5 μCi/100 g body wt) intraperitoneally. The radiochemicals were obtained from the Radiochemical Centre, Amersham, U.K. At the times indicated, rats were heparinized and killed by cardiac excision under light ether anaesthesia. Samples of blood were collected and frozen in liquid nitrogen. Initially, the rats were perfused through the ascending aorta with ice-cold physiological saline containing heparin [3] in order to eliminate any contribution from blood to tissue haem radioactivity. However, extremely low levels of haem radioactivity were found in blood, as has been reported previously [4], and in later experiments animals were not perfused. Similar results were obtained with and without perfusion.

The brain ventricles were opened and brain and liver samples were weighed, washed in chilled saline and frozen in liquid nitrogen. Tissues were maintained at −20° until analysis. Samples were thawed and homogenized in 3 vol. saline. Haem was extracted from the homogenate into ethyl acetate:glacial acetic acid (4:1, v/v) [5] and crystallized from the extract with the aid of carrier haemin [6]. Dried haem samples were prepared for determination of radioactivity by combustion in a Packard Sample Oxidizer.

Haem oxygenase activity in rat brain was measured by the method of Tenhunen *et al.* [7]. Tissue was homogenized in 4 vol. 0.1 M potassium phosphate buffer (pH 7.4) and centrifuged at 18,000 *g*. The reaction mixture (3.0 ml) contained 18,000 *g* tissue supernatant (6–9 mg protein), 17 μM haemin, 180 μM NADPH and 0.1 M potassium phosphate buffer (pH 7.4). In the control cuvette, NADPH was replaced by 0.1 M potassium phosphate buffer (pH 7.4). Hepatic haem oxygenase activity was assayed similarly, except that the NADPH concentration was 0.5 mM. The formation of bilirubin, determined from the increase in optical density at 468 nm, was linear for 10 min. Protein was determined by the method of Lowry *et al.* [8]. An extinction coefficient for bilirubin of 40 $mM^{-1}cm^{-1}$ was used [9]. Enzyme activity was expressed as nmoles bilirubin formed/10 mg supernatant protein/min.

Radioactivity incorporated into haem in rat brain fell rapidly between 6 and 16 hr after intraventricular injection of [4-^{14}C]ALA (Fig. 1). After 16 hr the decline was less rapid and by 48 hr the specific activity of brain haem had reached a plateau. The data of Schwartz [10] for the degradation of hepatic haem in dogs following intravenous injection of [4-^{14}C]ALA are also shown in Fig. 1 for comparison. Haem degradation in brain and liver apparently follow a similar time course up to about 24 hr after injection of [4-^{14}C]ALA. After 24 hr, radioactivity in hepatic haem continues to decline, whereas brain haem radioactivity remains relatively constant. The rates of brain and hepatic haem degradation in the 6–24 hr period following intra-

* ALA, δ-aminolaevulinic acid.